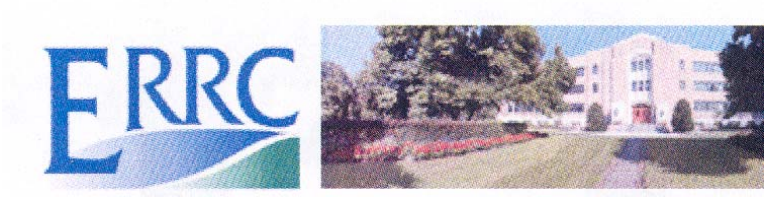

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Title: γ-Radiation Decontamination of Alfalfa Seeds Naturally Contaminated with *Salmonella* Mbandaka

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γ-Radiation Decontamination of Alfalfa Seeds Naturally Contaminated with *Salmonella* Mbandaka

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ABSTRACT: Samples of alfalfa seeds inoculated with *Salmonella* Mbandaka isolated from a naturally contaminated lot were γ-irradiated to 1 of 8 doses between 0 and 2.8 kGy, producing a maximum inactivation of 3.3 logs CFU/g. Analysis of the data indicates that the γ-radiation D value is 0.81 ± 0.02 kGy. An absorbed dose of 4 kGy, but not 3 kGy, eliminated viable *S.* Mbandaka from naturally contaminated seeds. Results of experiments with different percentages of inoculated seeds indicate that the dose required to inactivate a contaminating pathogen on alfalfa seeds is dependent upon the maximum contamination per seed, not the mean contamination of the lot.

Keywords: *Salmonella*, Mbandaka, γ-radiation, alfalfa, seed

Introduction

THE ASSOCIATION OF OUTBREAKS OF SALMONELLOSIS WITH THE consumption of contaminated sprouts grown from contaminated alfalfa seed is well established (National Advisory Committee on Microbiological Criteria for Foods (NACMCF) 1999; Proctor and others 2001; Taormina and others 1999; Winthrop and others 2003). Both chemical (for example, chlorine compounds, ethanol, hydrogen peroxide, ozone, and supercritical carbon dioxide) and physical (for example, ionizing-radiation, heat, and ultrasound) methods have been tested for the decontamination of seed that will be used for the production of food sprouts (Beuchat 1997; Beuchat and Scouten 2002; Mazzoni and others 2001; Scouten and Beuchat 2002, Sharma and others 2002, Thayer and others 2003). One of the physical methods under evaluation is the use of ionizing irradiation of the seed. The U.S. Food and Drug Administration (FDA) permits doses of ionizing radiation up to a maximum absorbed dose of 8 kGy for the purpose of controlling pathogens on seeds intended for sprout production (FDA 2000).

In our prior research on the quality of irradiated alfalfa sprouts and sprouts grown from irradiated seeds, we demonstrated increased content of ascorbic acid (Fan and Thayer 2001). Rajkowski and Thayer (2001) found that the keeping quality of irradiated alfalfa sprouts was double that of the nonirradiated sprout. These authors found that irradiation doses up to 5 kGy had little effect on the percent germination of the seeds when they were stored for periods of up to 6 mo following irradiation; however, the yield, or the grams of sprouts per gram of seeds, was lower when doses in excess of 2 kGy were used. Thayer and others (2003) determined that the irradiation D value of a mixture of *Salmonella* serovars, which included isolates associated with outbreaks of salmonellosis caused by the consumption of alfalfa sprouts, was 0.97 ± 0.03 kGy on sterile alfalfa seed. This D value was much higher than equivalent values reported for the inactivation of some of these same isolates on meat or poultry. These authors found that the inoculated bacteria were located predominantly on the surface of the seed near the hilum. The apparently high radiation resistance of these *Salmonella* cells did not appear to be caused by only the low moisture content of the seeds.

Frequently, analysts have been unable to isolate pathogens from seeds identified as the most likely source of contamination of food sprouts (NACMCF 1999). The NACMCF indicated that this suggests that the "contamination may be sporadic and at low levels." Van Benden and others (1999) analyzed the implicated seed lot during the 1995 to 1996 outbreak of *Salmonella* Newport and reported a contamination level of 4 to 24 cells/1000 g of seed. Similarly, Farrar and Mohle-Boetani (NACMCF 1999) determined that alfalfa seed implicated in a 1998 outbreak of salmonellosis contained approximately 4 CFU/1000 g. Gregory Inami (personal communication) analyzed the same lot (8119) of alfalfa seed that we used in the current study and isolated *Salmonella* Mbandaka at 1.8 MPN/100 g seed. Inami and Mohler (1999) compared different seed processing methods for the isolation of *Salmonella* from alfalfa seeds and concluded that the contamination level with the alfalfa seeds is not only low but is inconsistent within a sample.

The following study was conducted to determine the effectiveness of γ-irradiation in inactivating *S.* Mbandaka as a natural contaminant of alfalfa seed. The radiation resistance of *S.* Mbandaka on alfalfa seed has not been previously identified, nor has the radiation inactivation of a natural contaminant been studied.

Materials and Methods

Naturally contaminated alfalfa seed (lot 8119)

Two 25-kg bags of naturally contaminated alfalfa seeds (Lot 8119) were purchased from a U.S. seed distributor. Alfalfa sprouts grown from these seeds were linked to outbreaks of salmonellosis, caused by *Salmonella* Mbandaka, in Oregon, Washington, and Idaho in 1999 and were recalled by the distributor (Anonymous 1999; Inami and Moler 2001; Suslow and others 2002). Preliminary studies and the study reported in Table 1 were conducted with approximately 1 kg seeds aseptically removed from the top of 1 of the bags. It was assumed that commercial practices would ensure that the seed was well mixed and representative of the entire lot of seeds. Information from other laboratories caused us to question the consistency of the seeds in that 1 kg sample and resulted in the following effort to obtain a representative and consistent mix of seeds.

he remaining seeds (approximately 45 kg) were combined and septically mixed and stored within a decontaminated ice chest at °C. The ice chest was decontaminated with diluted bleach and hen rinsed with sterile water to remove the residual chlorine and llowed to dry before use. Microscopic examination (8 ×) of the eeds did not reveal evidence of scarification.

solation and identification of contaminant of alfalfa eed lot 8119

Two cultures of presumptive *Salmonella* were obtained by pre-nrichment of 375 g samples of Lot 8119 alfalfa seeds in lactose roth (Difco, Sparks, Md., U.S.A.), selective enrichment in brilliant reen (Sigma, St. Louis, Mo., U.S.A.) tetrathionate (BGTB) broth Difco), streaking on Hektoen Enteric agar (Difco) and, finally, treaking typical *Salmonella* colonies on tryptic soy agar (TSA) (Dif-o). All cultures were maintained and cloned on TSA. Culture iden-ity was confirmed by Gram stains and from reactions on the Gram-Negative Identification Cards of the Vitek AMS Automicrobic ystem (bioMérieux Vitek, Inc. U.S.A., Hazelwood, Mo., U.S.A.) (Al-lridge and others 1977, Knight and others 1990). Two *Salmonella* solates were submitted to the National Veterinary Service Labora-ories (Ames, Iowa, U.S.A.) for serotyping. Both isolates were typed as *S.* Mbandaka. Although *S.* Mbandaka was previously identified as associated with this lot of seed, the difficulty of its isolation and he inconsistency of the contamination made it essential for us to nsure that we could isolate the contaminate and confirm its iden-ity. One investigator had indicated to us that they thought that here might be more than one *Salmonella* serovar associated with his seed lot.

rradiation

The self-contained γ-radiation source (Lockheed Georgia Com-pany, Marietta, Ga., U.S.A.) has 23 ^{137}Cs pencils placed in an annu-lar array around a 63.5-cm high stainless steel cylindrical chamber with a 22.9 cm internal diameter. The source strength at the time of the study was ca. 117,355 Ci (4.34 PBq) with a dose rate of 0.10 kGy/min. The dose rate was established using alanine transfer dosim-eters from the National Institutes of Standards and Technology (Gaithersburg, Md., U.S.A.) Corrections for source decay were made monthly. Routine dosimetry was performed using 5-mm dia ala-nine dosimeters, sealed within 1.2 mL polypropylene cryogenic vials (Bruker Biospin Corp., Billerica, Mass., U.S.A.); the free-radi-cal signal was measured using an EMS 104 EPR Analyzer (Bruker Biospin Corp.) (ISO/ASTM 2002). Variations in sample dose ab-sorption were minimized by placing small samples within a uniform area of the radiation field, by irradiating the samples within a polypropylene container (4-mm wall) to absorb Compton electrons, and by using the same geometry for sample irradiation during each study. Under these conditions, the target dose and the actual ab-sorbed dose are the same within the limits of dosimetric measure-ment. This was confirmed by the routine dosimetric measure-ments. Based on measurement of dosimeter responses from several experiments, the actual doses were within ± 2% of the tar-get dose. The vials containing the dosimeters were taped to the sample pouches. Samples were maintained at 20 ± 1 °C during ir-radiation by the thermocouple-controlled injection of the gas phase from liquid nitrogen into the top of the irradiation chamber. Sample temperature was monitored continuously with thermocou-ples that were taped to 2 samples within the chamber.

Determination of radiation D value for *S.* Mbandaka

Seeds used for determination of radiation D values. Naturally contaminated alfalfa seed (Lot 8119) intended for use in the deter-mination of radiation D values and other studies requiring inocu-lation were packaged in 50 g amounts within heat-sealed Stomacher 400 full filter polyethylene bags (size: 175 × 304 mm, wall thickness: 65 μm, oxygen transmission: PO_2 5000 $cm^3/m^2/24$ h/ATM at 23 °C) (Seward, London, U.K.). The air was expressed by hand from the pouches, and then the pouches were heat-sealed. After packaging, the seeds were sterilized with a γ-radiation dose of 25 kGy at 20 °C. Typically, 10 pouches of seed were sterilized at a time.

Inoculation of sterile seeds. The following procedure was used to inoculate sterile seeds intended for use in studies to determine radiation D values. *S.* Mbandaka (SC1AXLT4B) (isolated from seed Lot 8119) was cultivated overnight in 100 mL of tryptic soy broth (TSB) (Difco) in a 500 mL Erlenmeyer flask at 37 °C on an orbital shaker at 150 rpm. A 50-mL inoculum of overnight stationary culture was mixed rapidly with 50 g of seed by hand massaging the pouch for 60 s. Immediately after mixing, the excess culture was decant-ed from the seeds (Thayer and others 2003). The filter from the bag containing the seeds was placed within a desiccator. The seeds were allowed to dry overnight in a vacuum over Drierite indicating 97% $CaSO_4$, $CaCl_2$ (WA Hammond Drierite Co. Ltd, Xenia, Ohio, U.S.A.) at room temperature. Two lots of inoculated seeds were pre-pared independently for 2 replicate studies. The average plate count for 2 replicate samples of dried inoculated seeds was $5.40 \times 10^8 \pm 2.92 \times 10^8$ CFU/g, and that of the inoculum was $9.9 \times 10^9 \pm 3.5 \times 10^8$ CFU/mL.

Determination of γ-radiation D values. Inoculated seed sam-ples were weighed (5.0 ± 0.1 g) and placed within the sample side of a Stomacher "side filter" polyethylene No. 400 pouch (size: 19 × 30 cm, wall thickness: 70 μm, oxygen transmission: 7 mL O^2/m^2 for 24 h) (Spiral Biotech, Norwood, Mass., U.S.A.). Each bag was heat-sealed after air had been expressed from it. Two bags of alfalfa seeds inoculated with *S.* Mbandaka were irradiated at 20 ± 1 °C per target dose. The target doses were 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 kGy. The nonirradiated controls were prepared and han-dled in exactly the same manner as the samples that were irradiat-ed. A dosimeter tube containing 5 alanine dosimeters was taped between each set of 2 sample pouches. Following irradiation, the pouches of irradiated seeds, including the 0-dose controls, were stored at room temperature overnight before analysis. Each pouch was opened at the top and 45 mL sterile Butterfield's phosphate buffer (BPB) was added aseptically and the mixture stomached for 90 s (Stomacher Model 400, Tekmar Co., Cincinnati, Ohio, U.S.A.). Serial dilutions were prepared with BPB, and appropriate dilutions were pour plated in triplicate using TSA. The Petri plates were incu-bated for 24 h at 37 °C before the average number of colony-form-ing units (CFU) on 3 Petri plates at the dilution producing 30 to 300 colonies was determined using an Accucount 1000 (Biologics, Gainesville, Va., U.S.A.). The study was replicated twice. The aver-age CFU/g of an irradiated sample (N) was divided by the average CFU/g of the nonirradiated controls (N_0) to produce a survival ra-tio (N/N_0). The D values (dose in kGy resulting in a 90% reduction of viable CFU) were the reciprocals of the slopes of linear regres-sions of the survivor ratio, as determined by least squares analysis. The zero-dose values were excluded from the calculation of the regression to avoid shoulder effects. Statistical calculations were performed with the general linear models procedure of the SAS sta-tistical package (Freund and others 1986; SAS 1987). The regres-sions were tested for differences by analysis of covariance.

Irradiation inactivation of *S.* Mbandaka in 25-g samples of nat-urally contaminated alfalfa seeds (Lot 8119). From the 1-kg sample of alfalfa seeds that had been obtained from the top of 1 of the 25-kg bags of Lot 8119, 18 subsamples (25 g) were aseptically prepared. Each 25-g sample was placed within the sample side of a Stomacher

Table 1—Survival of *Salmonella* Mbandaka in irradiated 25-g samples of naturally contaminated alfalfa seeds (lot 8119) after γ irradiation[a]

	Radiation Dose kGy					
Replicate	0	1	2	3	4	5
1	3/3	0/3	0/3	0/3	0/3	0/3
2	3/3	0/3	0/3	0/3	0/3	0/3
3	3/3	0/3	0/3	0/3	0/3	0/3

[a]Number of positive samples per 3 trials

"side filter" polyethylene No. 400 pouch. Each bag was heat-sealed after air had been expressed from it by hand. Three bags of alfalfa seeds, each 25 g, taken from the 1-kg subsample were irradiated per target dose. The target doses were 0, 1.0, 2.0, 3.0, 4.0, or 5.0 kGy at 20 °C. Following irradiation, each sample was diluted with lactose broth, 10-fold by weight, using an automatic gravimetric dilutor (Diluflo Model 800, Spiral Biotech). The samples incubated overnight at 37 °C. Aliquots of 1 mL were used to inoculate three 9-mL tubes of selenite cystine broth (Difco) and three 9-mL tubes of BGTB. The enrichment cultures were incubated overnight at 37 °C. A loopfull (3-mm platinum-iridium) from each tube after incubation was used to streak Hektoen Enteric agar. The Petri plates were incubated overnight at 37 °C. Blackish, dark green colonies were restreaked on TSA. Culture identity was confirmed by Gram reaction and from reactions on the Gram-Negative Identification Cards of the Vitek AMS Automicrobic System. If a *Salmonella* colony was identified from any 1 of 6 enrichment cultures per sample, it was listed as positive in Table 1. The study was replicated 3 times.

Irradiation inactivation of *Salmonella* Mbandaka in 15 × 25 g samples/replicate of naturally contaminated alfalfa seeds (Lot 8119). Lot 8119 was remixed, and 25-g pouches of seed were prepared as described above. After completion of the study described above, we became aware of the difficulty that analysts were having in confirming that particular alfalfa seed lots, including 8119, were the source of the outbreaks of salmonellosis. The results obtained by Inami and others (personal communication) suggested that statistically a composite sample of 375 g would be required to ensure

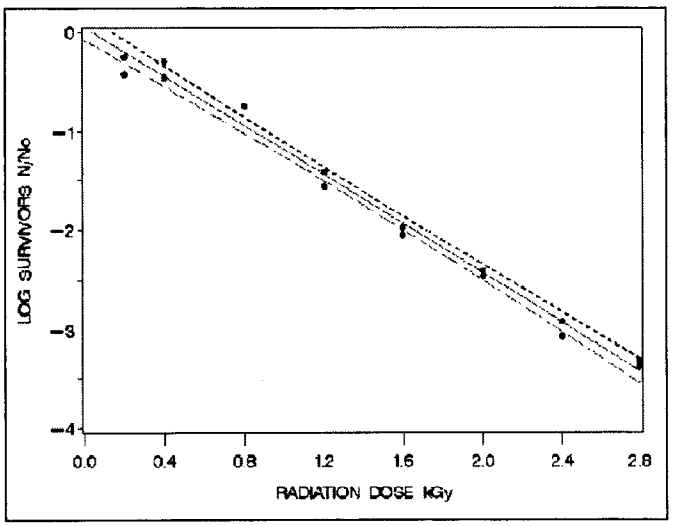

Figure 1—γ-irradiation inactivation of *Salmonella* Mbandaka on alfalfa seed. The predicted linear regression is illustrated by the solid line. The 95% confidence limits for the regression are illustrated by the dashed lines.

Table 2—Survival of *Salmonella* Mbandaka in Irradiated 15 × 25-g samples/replicate of naturally contaminated alfalfa seeds (lot 8119)[a]

Replicate	Medium	0 kGy	1 kGy	2 kGy	3 kGy	4 kGy	5 kGy
1	BGT	2/3	0/3	3/3	0/3	0/3	0/3
	SC	3/3	0/3	2/3	0/3	0/3	0/3
2	BGT	3/3	2/3	0/3	3/3	0/3	0/3
	SC	3/3	3/3	0/3	1/3	0/3	0/3
3	BGT	2/3	2/3	2/3	0/3		
	SC	3/3	0/3	1/3	0/3		
4	BGT	1/3	1/3	1/3	3/3		
	SC	0/3	0/3	3/3	2/3		
5	BGT	2/3	1/3	1/3	1/3		
	SC	2/3	0/3	0/3	1/3		
Positive Replicates		5/5	4/5	4/5	3/5	0/2	0/2

[a]Number of positive samples per replicate. BGT, brilliant green tetrathionate; SC, selenite cystine

isolation of *S.* Mbandaka. We elected to retest the seeds using composites consisting of 15 × 25-g bags of seeds per sample. As described, we pooled and mixed approximately 45 kg of alfalfa seeds from 2 bags (25 kg) from Lot 8119. A total of 450 samples (25 g) of seeds was prepared and heat-sealed within the sample side of a Stomacher "side filter" polyethylene No. 400 pouch. Each sample consisted of 15 bags (25 g) of alfalfa seed. The target doses were 0, 1.0, 2.0, 3.0, 4.0, or 5.0 kGy at a temperature of 20 °C. Following irradiation, each 25-g subsample was diluted with lactose broth 10-fold by weight, using an automatic gravimetric dilutor, and the samples incubated overnight at 37 °C. From each of the 15 pouches making up the composite sample, a 10-mL sample was withdrawn and pooled to form a single composite pre-enrichment sample. Aliquots of 1 mL were obtained from the composite pre-enrichment broth sample and used to inoculate 3 tubes (9 mL) of selenite cystine broth and 3 tubes (9 mL) of BGTB. The enrichment cultures were incubated overnight at 37 °C, and then a loopfull (3-mm platinum-iridium) from each tube was used to streak Hektoen Enteric agar. The Petri plates were incubated overnight at 37 °C. Blackish, dark green colonies were restreaked on TSA. Culture identity was confirmed by Gram stains and from reactions on the Gram-Negative identification cards of the Vitek AMS Automicrobic System. A replicate sample was considered positive if any colony was identified as *Salmonella.* The study was replicated 5 times.

Challenge study with sterile alfalfa (lot 8119) seed inoculated with *S.* Mbandaka.

The purpose of this study was a test of theory. If the inoculum is distributed uniformly on each seed and all seeds in the lot, then the dose required to inactivate all of the cells, when 100% of the seeds are inoculated, should be predictable from the D value. It would be very difficult to determine the between-seed variability in inoculum by counting because there are approximately 500 seeds/g. In a similar manner, if that seed sample were diluted by mixing with sterile seed, then equivalent reductions in the dose should be required to inactivate the pathogen in the seed mixture. Alternatively, if the radiation dose that is required to achieve inactivation is dependent upon the highest contamination level per seed, and not the average for the seed mixture, then the required dose will not decrease when the contaminated seeds are diluted with sterile seeds.

The same procedure as described above for the inoculation of sterile seeds with *S.* Mbandaka for the determination of D values was followed, except that the inoculum was diluted in BPB to pro-

vide an inoculum of approximately 1000 CFU/g seed. The actual inoculum of *S.* Mbandaka was determined by pour plate counts using TSA incubated at 37 °C for 24 h to be 930 CFU/g seed. Batches of inoculated seed were diluted by weight with sterile alfalfa seed to 20%, 30%, 40%, 60%, and 80%; total weight was 100 g or approximately 50000 seeds. Each sample was mixed well. The seed was packaged in 25-g amounts in No. 400 polyethylene Stomacher pouches as described above. Two 25-g pouches of seed were irradiated per dose. The target doses were 0, 1.0, 2.0, 3.0, and 4.0 kGy. Following irradiation, the samples were gravimetrically diluted 10-fold with lactose broth and incubated overnight at 37 °C. Aliquots of 1 mL were removed from each enrichment and used to inoculate 10 mL of TSB. The TSB cultures were incubated overnight at 37 °C, and then a loopfull was streaked on to TSA. Clones were selected from each Petri plate, and their identity as *Salmonella* was verified by Gram reaction and from reactions on the Gram-Negative identification cards of the Vitek AMS Automicrobic System. The study was replicated twice.

Results and Discussion

THE CONTAMINANT OF LOT 8119 OF ALFALFA SEED WAS CONFIRMED to be *Salmonella* and was identified serologically as *Salmonella* Mbandaka. These results confirmed identifications of this contaminant by Suslow and others (2002) and, more to the point, confirmed the identity of the organism that we tested.

The survival of irradiated *S.* Mbandaka isolated from the alfalfa seed Lot 8119 and reinoculated onto sterile seed is presented graphically in Figure 1. The radiation D value calculated from least-squares regression was 0.81 ± 0.02 kGy. The regression is described by the equation: log survivors = −1.241 kGy + 0.050 with $R^2 = 0.989$. Thayer and others (2003) found considerably higher D values of 0.98 ± 0.02 kGy for a cocktail of *Salmonella* isolates on alfalfa seed linked to outbreaks of salmonellosis. That cocktail consisted of *Salmonella* Anatum F4317, *Salmonella* Infantis F4319, *Salmonella* Newport H1275, and *Salmonella* Stanley H0558. Because D values for the individual isolates were not reported by Thayer and others (2003), it is unknown if any of those isolates were similar in radiation resistance to *S.* Mbandaka. The implication of a 0.81-kGy D value is that it leads to a prediction that a dose of 4.05 kGy should inactivate 5 logs of *S.* Mbandaka on alfalfa seed.

In preliminary studies, we were able to isolate salmonellae from a 25-g sample of alfalfa seed Lot 8119. The presumption then was that this lot of seed was severely contaminated. On the basis of these results, we designed an experiment in which 3 samples, 25 g of seed per treatment, were irradiated in Stomacher pouches at doses of 0, 1, 2, 3, 4, and 5 kGy. Following enrichment and selection of typical *Salmonella* colonies and identification as described in Materials and Methods, none was found in samples that had received a dose of 1 kGy or greater, and all 3 nonirradiated samples were positive (Table 1). On the basis of these data, one could conclude that a dose of 1 kGy should be adequate to eliminate *S.* Mbandaka from alfalfa seed and that the contamination of the seed was 1.23×10^1 CFU/g or lower.

Shortly after the completion of the above study, we became aware of data that indicated that contamination of this lot of seed was both very low and sporadic, requiring the use of larger samples to ensure its detection. As a result, we repeated the above study using 15 × 25 g composite samples of the seed per replicate, 5 replicates, and 2 separate enrichment media. The results of this study are presented in Table 2. Though all 5 of the nonirradiated samples were positive for *S.* Mbandaka, the results varied based on the enrichment medium used and radiation dose. In contrast to the results obtained in the 1st study, in the 2nd study samples were positive for *S.* Mbandaka that had received up to 3 kGy. Doses of 4 and 5 kGy eliminated the contaminant from the seed. These results imply that the contamination was very sporadic and did not exceed 10^5 CFU/g. Apparently, the results presented in Table 1 were due to chance, but are nevertheless important because comparison of the 2 studies demonstrates the sporadic type of contamination associated with this lot of seeds.

Table 3—Effect of the percentage of alfalfa seeds artificially inoculated[a] with *Salmonella* Mbandaka on the irradiation dose required to inactivate the pathogen

	Samples positive for *Salmonella*					
%Contaminated	0%	20%	40%	60%	80%	100%
0 kGy	0/2	2/2	2/2	2/2	2/2	2/2
1.0 kGy		2/2	2/2	2/2	2/2	2/2
2.0 kGy		1/2	2/2	2/2	2/2	2/2
3.0 kGy		2/2	1/2	1/2	1/2	2/2
4.0 kGy		0/2	0/2	1/2	2/2	1/2

[a]Inoculum 930 CFU/g

Table 3 presents the results of a challenge study in which sterile seed was inoculated with 930 CFU/g of *S.* Mbandaka. This seed was mixed with sterile seed and then irradiated. If the inoculum were uniformly distributed on all seeds, then a dose of 2.40 kGy (3 log × 0.81 kGy/log) would inactivate all of the cells when 100% of the seed was inoculated. In a similar manner, if that seed sample were diluted by mixing with sterile seed, then equivalent reductions in the dose would be required to inactivate the pathogen in the seed mixture. Alternatively, if the required radiation dose to achieve inactivation is dependent upon the highest contamination level per seed and not the mean for the seed mixture, then dilution of inoculated seed with sterile seed will not alter the dose required to eliminate *S.* Mbandaka. A radiation dose of 4 kGy failed to inactivate *Salmonella* Mbandaka at concentrations of 60% inoculated seed or greater, and the number of positive samples at 3 and 4 kGy indicates that the results may not be related to the percentage of inoculated seed. One can predict from the D value of 0.81 kGy for *S.* Mbandaka that a 4-kGy radiation dose should have produced a 4.9 log inactivation. This would seem to indicate that the contamination level on some of these inoculated seeds exceeded the mean inoculation level of 930 CFU/g. Perhaps some seeds were scarred and absorbed more of the inoculum. The number of seeds that had a higher contamination level could have been as few as 1 or as many as several hundred. Therefore, it is the maximum contamination per seed and not the mean contamination level that determines the required radiation dose.

Because our previous results (Rajkowski and Thayer 2001) demonstrated that satisfactory yields of sprouts could be obtained with alfalfa seed that had received an absorbed dose of 2 kGy, but not 3 kGy, we suggest that combination treatments using irradiated seed plus terminal seed treatments with agents such as calcium hypochlorite offer the best opportunity to reduce all pathogens by 10^5. Our results indicate that the maximum contamination with *S.* Mbandaka cells per seed of this naturally contaminated lot of alfalfa seeds did not exceed 10^5 CFU/g (Table 2).

Conclusions

THE RADIATION D VALUE FOR *SALMONELLA* MBANDAKA ON ALFALFA seed is lower than that of a mixture of *Salmonella* serovars on alfalfa seeds (Thayer and others 2003), and the pathogen can be eliminated by irradiating the seed. In the alfalfa seed Lot 8119, the distribution of the contaminant is sporadic. We conclude that, be-

cause the contaminant is sporadically distributed on the seed, the dose that will be required for inactivation will reflect the spot and not the mean concentration of the contaminating organism.

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